# Effects of tooth-brushing force with a desensitising dentifrice on dentine tubule patency and surface roughness

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# Abstract

Objectives: To investigate the effects of a 5% NovaMin containing dentifrice on dentine tubule patency and surface roughness at 100g and 400g tooth brush abrasion forces.

Methods: 75 polished human dentine samples were prepared and randomly allocated into one of five groups; control (1), $Na_2PFO_3$ 100 g abrasion force (2), NovaMin 100 g (3), $Na_2PFO_3$ 400 g (4) and NovaMin 400 g (5). The control group underwent two 2-minute cycles of artificial saliva (AS), one 2-minute erosion cycle; the rest underwent two toothbrush abrasion cycles in an AS/dentifrice slurry and one 2-minute erosion cycle. All samples were imaged at baseline and post intervention using Tandem Scanning Microscopy and Profilometry to analyse tubule patency and roughness.

Results: Mean tubule patency increased significantly between baseline and post intervention in groups 1,2 and 4 and decreased significantly post intervention in groups 3 and 5 ($p<0.01$). Post intervention, there were statistically significant differences in mean patent tubules between NovaMin and the $Na_2PFO_3$ and control groups ($p<0.001$). Surface roughness increased for all groups between baseline and post interventions ($P<0.001$); mean (SD) roughness increases for groups 1, 2, 3, 4 and 5 were 0.14 (0.05) µm, 0.18 (0.04) µm, 0.16 (0.06) µm, 0.19 (0.07) µm and 0.21 (0.02) µm respectively. Differences between group 1 and 5 were significant ($p< 0.01$).

Conclusions: Brushing with NovaMin resulted in significant dentine tubule occlusion at 100g and 400g, but brushing with $Na_2PFO_3$ resulted in increased tubule patency. Surface roughness increased significantly at 400g brushing with NovaMin. There was no correlation between tubule patency and surface roughness.

Clinical significance: A NovaMin desensitising dentifrice resulted in tubule occlusion even at high brushing forces. There was minimal increase in surface roughness at the lower (100g) brushing force.

# Introduction

Dentine hypersensitivity is defined as a short duration, sharp dental pain response to stimuli in the absence of any other pathology [1,2]. The generally accepted mechanism behind dentine hypersensitivity is the hydrodynamic theory [2,3]. This involves the rapid transmission of fluid through dentinal tubules triggering neuroreceptors located in the pulp in response to stimuli such as cold and air [4]. The condition is very prevalent in Europe (42%) and especially the UK [5][6]. Diet [7] as well as tooth-brushing [8][9] are important aetiologies.

In order for the fluid flow in the hydrodynamic theory to be possible, the dentinal tubules must be patent (open). Studies have identified that teeth diagnosed with dentine hypersensitivity possess a greater number of patent tubules on the dentine surface [10,11]. It is therefore not surprising that the number of patent tubules on the dentine are used to assess the efficacy of desensitising (tubule occluding) products [12]. Olley *et al*. 2014 developed a robust reproducible method to quantify patent tubules in dentine samples. Dentine samples were scanned using Tandem Scanning Microscopy (TSM) and the images analysed using a software program to quantify the number of patent dentinal tubules [13]. This method was used in a further study investigating the effects of three toothbrush abrasion forces on tubule patency using a standard $Na_2PFO_3$ toothpaste [9]. This study reported an association between increased tubule patency and increased abrasion after one erosion-abrasion cycle, with significant differences at the 100g and 400g abrasion forces [9]. However, the effects of a densensitising (tubule occluding) dentifrice at these brushing forces (100g and 400g) and whether the higher brushing force could increase tubule patency despite the dentifrice are unknown. This study investigates if there is a protective effect (reduced tubule patency) of a desensitising dentifrice (5 % NovaMin, GlaxoSmithKline Consumer Healthcare, Brentford, UK) at both 100g and 400g toothbrush abrasion forces in an erosion/abrasion regime. NovaMin is a bioactive glass with calcium sodium phosphosilicate as the active ingredient. It is reputed that this can react in the oral environment to form a hydroxy-carbonate apatite (HCA) over time and is similar to the natural tooth mineral

composition [14–16]. It has been previously shown when comparing two NovaMin containing dentifrices, control and water, that the NovaMin results in dentine tubule occlusion [13].

It should not be supposed from this study that the effect of a desensitising dentifrice might offset the detrimental effect to dentine at higher brushing forces and therefore enable this to occur. The 400g brushing force is represented as an overzealous regime to investigate the effects on the dentine surface.

In addition, the measurement of surface topography is widely used within dental material science, with rapidly evolving developments [17]. Surface roughness measurements are often used to identify changes in tooth structure following erosive wear and to investigate the efficacy of anti-erosion and remineralising products [18–21]. Furthermore, tribology studies use roughness parameters to make associations between wear patterns and diet [22,23]. To the authors' knowledge a correlation between surface roughness and tubule patency has not been investigated. It can be supposed that a change in the tubule patency of dentine, could effect the surface roughness of dentine due to the surface nature of dentine hypersensitivity [24]. Therefore surface roughness may prove a useful indicator of tubule patency.

Therefore, the overall aim of this study was to investigate the effects of a 5% NovaMin containing dentifrice on dentine tubule patency and surface roughness at two abrasion forces (100g and 400g). The null hypothesis was that there would be no difference in tubule patency and surface roughness brushing with a NovaMin containing dentifrice at 100g and 400g tooth-brushing forces.

# Methods

## Sample preparation

Unrestored and caries free human molars were collected under ethical agreement (12/LO/1836) and sterilised in sodium hypochlorite for a minimum of 72 hours. The roots were removed and the crowns sectioned using a circular diamond saw (XL 12205, Benetec Ltd., London, UK) to produce 75 sections, no samples were discounted during the study. The sections were embedded in bisacryl composite

(Protemp4 3M ESPE, Germany) using custom made trays to make samples. Sample size calculations were based on Sehmi and Olley *et al.* 2015. Samples underwent a standardised polishing regime using a series of carbide grits (320, 1200, 2400 and 4000) in a water cooled polishing machine (Meta-Serv 3000 Grinder-Polisher, Buehler, Lake Bluff, Illinois, USA) to produce areas of optically flat dentine with a flatness tolerance of 0.4 µm [25]. This process created an artificial smear layer on the surface of dentine (based on the protocol from Sehmi and Olley *et al.* 2015). This layer was removed following the first brushing (in the next stage of the experiment) by immersing the samples in citric acid to create an etching effect.

## Experimental design

The 75-dentine samples were randomly allocated into one of five groups, with 15 samples per group. Group 1 was the "control group"; these samples did not undergo any toothbrush abrasion or exposure to dentifrice. Control samples were immersed in artificial saliva (AS) for 2 minutes followed by immersion in 0.3% Citric Acid pH 2.6 for 2 minutes and completed by immersion in AS for a further 2 minutes. The remaining groups compared two dentifrice products, Colgate Cavity Protection (Colgate Oral Pharmaceuticals, New York, USA) ($Na_2PFO_3$) and Sensodyne® Repair & Protect (5% NovaMin) (Calcium Sodium Phosphosilicate). Each dentifrice was investigated at two abrasion forces; 100g and 400g.

The dentifrice slurries were made immediately before use and consisted of 1-part dentifrice (330 ml) to 2-parts AS (660 ml) and hand-mixed for 2 minutes. The AS was made used within 24 hours following an established protocol and consisted of Calcium Chloride Dehydrate 0.7 mmol/l, Magnesium Chloride 0.2 mmol/l, Potassium Dihydrogen Phosphate 4.0 mmol/l, HEPES (Acid buffer) 20.0 mmol/l and Potassium Chloride 30.0 mmol/l and buffered to pH7 [26]. A reciprocating and automatic tooth brushing machine (Dentagen, Munich, Germany) with standardised toothbrushes (Sensodyne® Search 3.5 with small head sizes) was used for the abrasion experiments. To achieve the desired abrasion force (100g or 400g) external calibrated weights (Voltcraft PS 500 Pocket scale, Oldenzaal, Netherlands) were attached and the force applied to the tip of each toothbrush to engage on the

centre of each dentine sample. The dentine samples were fully immersed in the toothpaste/AS slurries in reservoir baths located in the tooth brushing machine, which was thoroughly cleaned between groups. The dentine samples were abraded in dentifrice slurry at their designated abrasion force for 2 minutes (120 strokes) using a soft bristled tooth brush, followed by immersion in 0.3 % Citric Acid pH 2.6 agitated at room temperature for 2 minutes and followed by a further 2 minutes dentifrice abrasion (as per Sehmi and Olley 2015 [9]).

## TSM imaging

TSM imaging and analysis were carried out at baseline and post experimental intervention by the same operator. The samples were rehydrated for a minimum of 5 hours in phosphate buffered (pH 7) distilled water prior to imaging with the TSM (Noran instruments, Middleton USA) using an M-plan 40x SLWD (Brightfield Objective x 40/0m35 NA objective). Gently air dried samples were placed on a platform at the microscope and imaged on the TSM machine digitally using a mounted camera (Andor iXon 885, Andor Technology Ltd, Belfast, UK) with iAndor software. The TSM light source was positioned over the centre of the dentine samples; the adjacent composite in the mount was marked to reliably relocate the same area after the experimental intervention. A previously validated computer algorithm (Image J software, USA) was used to count the number of patent dentine tubules greater than 0.83µm [9].**Error! Reference source not found.**

## Surface roughness

All of the samples were imaged and analysed for surface roughness by the same operator who was randomised to active ingredient. Scanning was carried out using a non-contact profilometer (NCP) with a red laser light source (2 µm spot size; NCP, LT-9010M, Keyence Corporation, Japan) and motion controlled stage (Xyris 2000, Taicaan, UK). MountainsMap (DigitalSurf, France) analysis software was used to extract Sa roughness (average roughness of a measured surface) following application of a 25 µm Gaussian filter. Five randomly selected areas (each 0.04 $mm^2$) within the centre of the dentine samples were imaged and analysed before and after experimental intervention.

### Statistical analysis

The sample size for this study was based upon a power calculation used in previously published study and pilot work ([9]) with an alpha level of 0.05, 80 % power, mean patent dentine tubules 180 and standard deviation 50 [10,9,27]. Shapiro-Wilk and Kolmogorov-Smirnov tests, along with histogram plots were used to determine the normality of the data. The data were found to be normally distributed. Levene's tests were performed to assess homogeneity of variances; TSM data had equal variance therefore a two way ANOVA and post hoc Tukey test were used to determine inter and intra group significance. However, surface roughness data did not have equal variance and in this case a Welch ANOVA and post hoc Games-Howell test were used. Pearson correlation tests were used to examine the relationship between dentinal patency and surface roughness.

## Results

### TSM

The mean patent tubules at baseline and post intervention for all groups are shown in Table 1. Between baseline and post intervention, there were statistically significant increases in dentine tubule patency in the control and $Na_2PFO_3$ groups whereas there were significant decreases in tubule patency for the NovaMin groups. For group 1, control, the mean (SD) number of dentine tubules at baseline was 188 (60) which statistically significantly increased to 245 (49) post erosion ($p< 0.01$). For group 2 (100g abrasion force with $Na_2PFO_3$ dentifrice), the mean (SD) number of dentine tubules at baseline was 193 (48), which statistically significantly increased to 238 (47), post erosion-abrasion ($p< 0.01$). Group 3 (100g abrasion force with NovaMin dentifrice), had a baseline mean (SD) of 185 (63) which statistically significantly decreased to 146 (41) post erosion-abrasion ($p < 0.001$). Group 4 (400g abrasion force with $Na_2PFO_3$ dentifrice), had a baseline mean (SD) of 185 (44) which statistically significantly increased to 253 (48) post erosion-abrasion ($p < 0.001$). Group 5 (400g abrasion force with NovaMin dentifrice), had a baseline mean (SD) which statistically significantly decreased from 201 (42) to 133 (63) post erosion-abrasion $p<0.001$. Representative images for each group are shown in Figure 1; images were numbered in accordance with their group with image A at baseline and image B at post intervention. At baseline, there were few visible patent dentine tubules in each image for groups

1-5. Increased numbers of visible patent tubules post intervention were identified in the associated images in groups 1, 2 and 4. There were numbers of visible patent tubules post intervention in groups 3 and 5 and the surface appears to be occluded compared to baseline. Inter group comparisons revealed no statistically significant differences between groups at baseline. At post intervention there were statistically significant differences in the number of patent tubules between the NovaMin 100g abrasion force and control, the $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces; the NovaMin 400g abrasion force compared to control and the $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces. These findings are summarised in table 2

### Surface roughness

In all groups, there was a significant increase in surface roughness post intervention, $p< 0.001$. The mean roughness change and standard deviations for each group are shown in the graph in Figure 2. The mean (SD) of roughness change for group 1, 2, 3, 4 and 5 were 0.14 (0.05) µm, 0.18 (0.04) µm, 0.16 (0.06) µm, 0.19 (0.07) µm and 0.21 (0.02) µm respectively with a statistically difference between group 1 (control) and group 5 (NovaMin at 400g brushing force) $p<0.01$.

### Correlation between tubule patency and surface roughness

There was no correlation between surface roughness and tubule patency results. The correlation between patent dentine tubules and surface roughness between all samples at baseline was 0.2. The correlation between all samples post intervention was 0.02. When comparing the change in roughness and change in patent dentine tubules (between baseline and control for all samples), the correlation was 0.11.

## Discussion

This study showed that a 5% NovaMin desensitising dentifrice significantly decreased tubule patency post acid challenge at both 100g and 400g abrasion forces. There was a significant difference in roughness change with the 5% NovaMin at 400g compared to control, but no significant difference in roughness change using the 5% NovaMin at 100g or the $Na_2PFO_3$ dentifrice at 100g and 400g.

However, there were no statistically significant direct associations between DH and surface roughness. Thus the null hypotheses can be refuted for the former but not for the latter.

There is clinical importance to investigate the effects of various brushing force. Brushing force will vary throughout an everyday brushing regime and various individuals will use different brushing forces [28]. Ganss *et al*. 2008 conducted a study on 108 participants to investigate tooth-brushing habits including measuring force applied. In their study they reported the mean force applied to be 235g with a maximum of 480g [29]. Wiegand *et al.* 2014 investigated a smaller participant group which had been given specific tooth brushing instructions and reported a mean force between 92g using sonic toothbrushes and 163g using manual brushes [30]. It is accepted that clinically brushing forces will vary, however, for the purposes of *in vitro* studies low brushing force is established as 100g and high brushing force is established as 400g [31]. This current study progressed from work by Sehmi *et al*. 2015, which compared three brushing forces; 100g, 200g and 400g (low, medium and high). Significant differences in tubule patency occurred at 100g and 400g brushing forces [9]. At 100g they identified the formation of a smear layer post tooth brush abrasion (and erosion), but at 400g they identified significant increases in tubule patency which is clinically relevant for patients with DH [9]. Therefore, we aimed to investigate what potential therapeutic effects a NovaMin containing dentifrice would have using erosion-abrasion regimes at these brushing forces (100g and 400g). The decision to use 400g was based upon the findings in the previous study and by no means was meant to encourage using this force at a clinical level. In addition, the use of citric acid to remove smear layer is well documented and is an important dietary erosive factor in tooth wear and dentine hypersensitivity, often in combination with overzealous tooth brushing [32] [9] [33]. The brushing time of two minutes was chosen based upon Public Health England recommendations for the whole mouth [34]. Understandably a single tooth surface would only receive a proportion of this in one sitting not the full two minutes, however this study represents long term brushing at these forces (over 6-8 weeks). Furthermore, this is the same brushing duration as the previous study investigating brushing force, to enable comparison [9].

The control and the two $Na_2PFO_3$ dentifrice groups demonstrated a statistically significant increase in the number of patent dentine tubules recorded between baseline and post erosion-abrasion interventions. More patent tubules were recorded post erosion-abrasion as the abrasion force increased from 100g to 400g and this is similar to previously published work [9]. However, the NovaMin dentifrice groups demonstrated a significant decrease in patent tubules at 100g and 400g brushing forces. This has a particular clinical benefit for DH [12]. The clinical effects of the prophylactic use of NovaMin paste have been explored in a blinded randomised controls trial by Olley *et al* 2014 [13] and Neuhaus *et al* 2013. The latter assessed dentine hypersensitivity following applications of NovaMin pastes using tactile and air stimuli as well as a participant questionnaire [35]. It was suggested that a single application of a NovaMin based product could be enough to significantly decrease dentinal hypersensitivity [35]. The decrease in patent tubules in our study also supports findings from previous studies, which used different methodologies. Wang *et al*. 2010 investigated the permeability of dentine and SEM to quantitatively and qualitatively assess the effects of abrasion with NovaMin and identified that the permeability of dentine decreased. This occurred as a result of occlusion of patent dentine tubules [36]. In another *in vitro* study, which investigated the effects of NovaMin on tubule occlusion with blinded assessors scoring SEM images using a visual scale, Chen *et al*. 2015 demonstrated that treating the surface with NovaMin resulted in tubule occlusion [37]. The benefit of using the computer algorithm in our study compared to using a visual scale is that it removes any operator bias or inter operator reproducibility issues making it a robust and reliable quantitative tool [13]. Olley *et al*. 2014 compared two occlusion-causing dentifrices (both were NovaMin based) against a standard $Na_2PFO_3$ dentifrice (control). They used the computer algorithm along with more traditional scoring methods to quantify tubule occlusion. The computer algorithm was able to detect tubule patency at an increased level compared to the naked eye. Images, which were graded as completely occluded by visual scoring system, were found to have patent tubules that could cause dentine hypersensitivity, using the computer algorithm. In their study the dentifrices investigated resulted in significant tubule occlusion over the four day *in situ* study, compared to control [13].

However, Olley *et al.* 2014 did not investigate NovaMin at controlled values of brushing force at 100g and 400g. At both 100g and higher 400g brushing forces, the present *in vitro* study demonstrated tubule occlusion also. There was a larger decrease in patent tubules when the 400g-abrasion force was applied, in contrast to the 100g-abrasion force with NovaMin. However, the differences between 100g and 400g brushing with NovaMin on the tubule patency were not significant and therefore should be interpreted as no difference. Sehmi *et al*. 2015 used the computer algorithm to investigate tubule patency in TSM images at multiple stages of their erosion-abrasion regime and suggested that brushing with a 400g force could have a role in removing the smear layer [9]. By removing the smear layer, we can suppose (but not prove) that this might create a better scaffold for NovaMin uptake. However, it would not be recommended to clinically apply a 400g brushing force as the increased risk of wear at this force would be counterintuitive to the therapeutic effect of the NovaMin product.

There was a statistically significant difference in roughness change between group 1 (control) and group 5 (NovaMin group at 400g abrasion force). Thus, the higher brushing force produced a statistically rougher surface using the desensitising dentifrice. In contrast, when smaller brushing forces (100g) were applied with the 5% NovaMin, the increase in surface roughness was the smallest reported in any group other than control (not statistically significant). Therefore, there does seem to be an effect of brushing force on the surface roughness and the 5% NovaMin produced a relatively smoother surface when used at the smaller (100g) brushing force. One possible explanation for this relates to how Sa roughness is calculated. Surface roughness is height deviation from the form or overall shape, of a surface [38][39,40]. A limitation of Sa is that it provides a quantitative mean of the height deviations and cannot differentiate if there is loss or gain (pits or valleys) [38]. In the case of the NovaMin we understand from our TSM images in  and previous studies that interaction with NovaMin products results in a superficial layer of hydroxyapatite on the dentine surface, which also occludes the dentinal tubules [36,37]. Following this theory, the artificial layer created at the 100g brushing force with NovaMin may have reduced the height deviations into the exposed patent dentine tubules and produced relatively little increases in surface roughness between baseline and post

intervention. In contrast, at the 400g brushing force with NovaMin, there were increased height deviations. One theory is that this is due to the improved uptake and deposition of more surface layer, as described in the paragraph above. Therefore, the increase in surface roughness at 400g with NovaMin was greater than at 100g. In the $Na_2PFO_3$ and control groups, more tubules were patent post interventions (compared to baseline) with little surface deposit; and the increased surface roughness was related more to exposure of patent dentinal tubules as opposed to uptake of surface product. It could be suggested that the type of dentifrice used is likely to affect roughness. In effect, another theory as to why the surface roughness increased most at 400g with desensitising dentifrice (and 240 brush strokes) might be related to the higher abrasivity of the desensitising dentifrice on the dentine surface itself [20].

With the various dentifrices and toothbrush regimes, it is therefore not surprising that despite the statistical differences noted for both tubule patency and surface roughness results, there was no direct correlation between the surface roughness and tubule patency across all samples. Although surface roughness was not a direct indicator of tubule patency in this study, surface roughness measurements were useful to help formulate an understanding of the effects of tooth brushing force and dentifrice on the dentine surface as described. Indeed, surface roughness has also been used in previous studies to help differentiate the nature of wear patterns on enamel and dentine [17,23][21].

## Conclusion

Brushing with 5% NovaMin containing dentifrice resulted in significant dentine tubule occlusion at both 100g and 400g abrasion forces. Surface roughness only increased significantly at 400g toothbrush abrasion force with NovaMin; there was minimal increase in surface roughness at 100g brushing. There was no correlation between tubule patency and surface roughness.

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# Tables

*Table 1: Mean (SD) number of patent tubules before and after intervention for all 5 experimental groups; group 1 control, group 2 100g abrasion force $Na_2PFO_3$, group 3 100g abrasion force NovaMin, group 4 400g abrasion force $Na_2PFO_3$ and group 400g abrasion force NovaMin.* Intra group statistics are noted * = P< 0.05 ** = P< 0.01 *** = P< 0.001

| **Experimental Group** | **Mean (SD) patent tubules before intervention** | **Mean (SD) patent tubules post intervention** |
|---|---|---|
| **Control** | 187.60 (60) | 244.60 (49) ** |
| **Na2PFO3 100g** | 193 (48) | 238 (47) ** |
| **NovaMin 100g** | 185 (63) | 146 (41) *** |
| **Na2PFO3 400g** | 185 (44) | 253 (48) *** |
| **NovaMin 400g** | 201 (42) | 133.27 (63) *** |

Table 2: Inter group significant differences between number of tubules recorded from baseline to post intervention expressed in Mean Difference (MD) and standard error (SE). There were significant differences in MD between NovaMin 100g abrasions force and control, $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces. There were also significant differences MD between NovaMin 100g abrasions force and control, $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces. Inter group statistics are noted * = P< 0.05 ** = P< 0.01 *** = P < 0.001

| | **NovaMin 100g** | **NovaMin 400g** |
|---|---|---|
| **Control** | MD = +/-107***<br>SE = 18 | MD = +/-111***<br>SE = 18 |
| $Na_2PFO_3$ **100g** | MD = +/- 92***<br>SE = 18 | MD = +/-105***<br>SE = 18 |
| $Na_2PFO_3$ **400g** | MD = +/-107***<br>SE = 18 | MD = +/-120 ***<br>SE = 18 |

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# Tables

*Table 1: Mean (SD) number of patent tubules before and after intervention for all 5 experimental groups; group 1 control, group 2 100g abrasion force $Na_2PFO_3$, group 3 100g abrasion force NovaMin, group 4 400g abrasion force $Na_2PFO_3$ and group 400g abrasion force NovaMin.* Intra group statistics are noted * = P< 0.05 ** = P< 0.01 *** = P< 0.001

| **Experimental Group** | **Mean (SD) patent tubules before intervention** | **Mean (SD) patent tubules post intervention** |
|---|---|---|
| **Control** | 187.60 (60) | 244.60 (49) ** |
| **Na2PFO3 100g** | 193 (48) | 238 (47) ** |
| **NovaMin 100g** | 185 (63) | 146 (41) *** |
| **Na2PFO3 400g** | 185 (44) | 253 (48) *** |
| **NovaMin 400g** | 201 (42) | 133.27 (63) *** |

Table 2: Inter group significant differences between number of tubules recorded from baseline to post intervention expressed in Mean Difference (MD) and standard error (SE). There were significant differences in MD between NovaMin 100g abrasions force and control, $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces. There were also significant differences MD between NovaMin 100g abrasions force and control, $Na_2PFO_3$ 100g and $Na_2PFO_3$ 400g abrasion forces. Inter group statistics are noted * = P< 0.05 ** = P< 0.01 *** = P < 0.001

| | **NovaMin 100g** | **NovaMin 400g** |
|---|---|---|
| **Control** | MD = +/-107***<br>SE = 18 | MD = +/-111***<br>SE = 18 |
| $Na_2PFO_3$ **100g** | MD = +/- 92***<br>SE = 18 | MD = +/-105***<br>SE = 18 |
| $Na_2PFO_3$ **400g** | MD = +/-107***<br>SE = 18 | MD = +/-120 ***<br>SE = 18 |

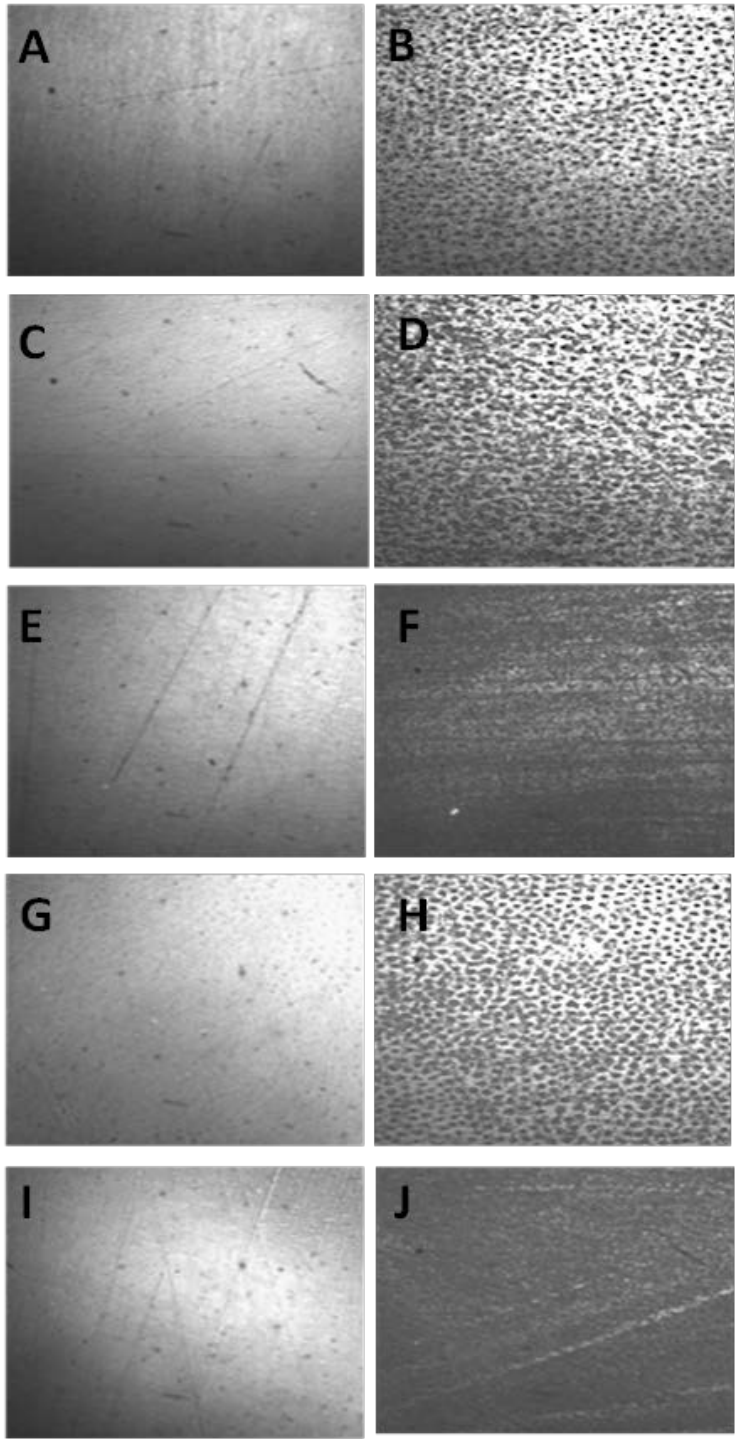


Figure 3: Representative TSM images for each group at baseline and after Intervention. There was an increase in tubule patency visible in Group 1 (control) labelled A & B, Group 2 (Na2PFO3 100g) labelled C & D and Group 4 (Na2PFO3 400g) labelled G & H whilst tubule occlusion was visible in Group 3 (NovaMin 100g) labelled A & F and Group 5 (NovaMin 400g) labelled I&J)